# THE PREPARATION OF DRIED CULTURES.*†

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## INTRODUCTION.

The use of cultures in the dairy industry is of ancient origin, altho it is only in very recent years that the real nature of the cultures has been understood. While specially prepared cultures are now used in many creameries and even in therapeutics, a faulty technic has prevented the production of cultures of the highest purity and activity and has retarded their commercial development. All of the better class of creameries use pure cultures which are obtained at regular intervals from commercial laboratories. These are carried in the creameries by transferring milk cultures from day to day, the culture being renewed occasionally to insure its purity. The small milk culture known as a "mother starter" is usually carried in bottles or small jars, from which it is transferred to a large can or vat of milk to make the starter used to ripen the cream.

The ideal culture for distribution is in a dry form, sufficiently active to produce rapid growth when it is added to milk and yet so dormant that it can be held a long time without losing its activity. But the difficulties of producing a culture in this condition are so great that the most successful of the commercial cultures are distributed in a liquid medium and must be used within a comparatively short time. A few cultures, however, have been sold continuously in a powder or tablet form; in other cases the liquid culture has been found to be more satisfactory and the dry culture has been abandoned.

Recently the general interest in fermented milks, especially those of the yogurt type, has stimulated the production of various tablets and capsules which ostensibly contain the organism in such quantities that the culture can be used to start a fermentation in milk or to inoculate the digestive tract by direct consumption.

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These cultures, as well as those sold for butter-making and cheese-making, are of real value only when they contain a suitable organism free from contamination and are sufficiently active to start the acid fermentation before accidental contamination can develop to an appreciable extent.

### CONDITION OF COMMERCIAL DRY CULTURES.

The poor quality of the ordinary yogurt tablet has been discussed in a previous paper.[1] Very few of the cultures examined in this laboratory have contained enough *B. bulgaricus* to sour milk before a large development of contaminating bacteria had occurred, and in many cases the contamination was so great that it was difficult to find the acid-forming organism at all. The butter cultures sold in the dry form are powders or tablets sometimes prepared with a filler of starch or lactose, or are milk cultures dried and pulverized.

TABLE 1.

SHOWING CONDITION OF COMMERCIAL CULTURES.

| Culture | Bacteria per Gram | Contamination |
|---|---|---|
| A1 | 69,300 | 12 per cent |
| A2 | 492,700 | Slight |
| A3 | 17,400 | " |
| A4 | 6,520,000 | " |
| B1 | 770,000 | ..... |
| B2 | 89,500 | 42 per cent |
| B3 | 365,000 | Very high |

The condition of various samples of cultures is shown in Table 1. One of these cultures, *A*, was a tablet, while *B* was evidently a dried-milk culture.

In preparing starters in accordance with the directions which accompany these cultures, a considerable portion of the powder is added to pasteurized or boiled milk. When this has curdled a small portion is transferred to fresh milk, and the process is repeated until a curd of good flavor and free from evidences of contamination is obtained. The result of this manipulation is the elimination of the contamination, usually very evident in the first transfer.

The rate of development of dried cultures when added to sterile milk and incubated at 30° C. is shown in Table 2. Culture *A* was

[1] *Circular 171*, U.S. Dept. of Agric., Bureau of Animal Industry, Washington, 1911.

a tablet, *B* and *C* were powders, while *D* was not a commercial culture but a powder prepared at our request by a special process which will be considered later.

In the commercial cultures examined nearly all of the bacteria had been destroyed in the preparation, and in one case the culture was almost sterile. It is obvious that in the ordinary process of drying cultures there is a great decrease in the number of bacteria originally present in the culture, and it is probable that there is a still further loss as the culture is held after drying.

TABLE 2.

CULTURES MADE FROM COMMERCIAL POWDERS.

| CULTURE | RATIO OF POWDER TO MILK | BACTERIA PER GRAM OF POWDER | MILK AFTER 8 HRS. AT 30° C. | | MILK AFTER 19 HRS. AT 30° C. | |
|---|---|---|---|---|---|---|
| | | | Acidity | Bacteria per c.c. | Acidity | Bacteria per c.c. |
| | | | Percentage | | Percentage | |
| A..... | 1:200 | 0 | .216 | 0 | .261 | 0 |
| B..... | 1:200 | ..... | .261 | 103,500 | .855 | 1,275,000,000 |
| C..... | 1:200 | ..... | .225 | 101,500 | .819 | 90,500,000 |
| D..... | 1:200 | 6,120,000,000 | .819 | 1,895,000,000 | 1.071 | 1,280,000,000 |
| A..... | 1:1000 | ..... | .270 | 12,000 | .279 | 9,300,000 |
| B..... | 1.1000 | 3,650,000 | .261 | 1,150,000 | .684 | 1,705,000,000 |
| C..... | 1:1000 | ..... | .252 | 2,000 | .270 | 34,500,000 |
| D..... | 1:1000 | 8,590,000,000 | .387 | 223,000,000 | .855 | 1,840,000,000 |
| A..... | 1:500 | 69,300 | .207 | 47,750,000 | .414 | 322,000,000 |
| B..... | 1:500 | 89,500 | .203 | 2,230,000 | .819 | 635,000,000 |
| C..... | 1:500 | ..... | .198 | 15,000 | .684 | 135,500,000 |
| D..... | 1:500 | 2,015,000,000 | .342 | 526,500,000 | .999 | 1,710,000,000 |

## DECREASE OF BACTERIA IN DRYING.

A freshly curdled milk culture of the ordinary lactic acid bacteria usually contains about 1,000,000,000 bacteria per c.c. If this were reduced to a water-free powder each gram would contain approximately 100,000,000,000 bacteria. The usual process of drying cultures consists in reducing the percentage of water by adding a dry substance, as for instance, powdered lactose, and exposing the mixture to a current of air at a temperature sufficiently high to cause a rapid evaporation. In order to insure a reasonably rapid loss of water it is necessary to hold the culture at a temperature somewhat above the optimum, and, with some cultures, above the upper temperature limit of growth. As the drying progresses, there is a concentration of the acid which there is every reason to believe would

have a detrimental effect on the bacteria. There is also a concentration of other solids in solution in the water of the culture, and there must come a point when the concentration of the solids in the water surrounding the cells becomes so great that a large part of the water is withdrawn by the osmotic pressure and plasmolysis results.

Table 3 shows the moisture and bacterial content of a culture at half-hour periods during the drying. The apparatus in which this preparation was made consisted of a water oven, through which was blown air previously dried by passing over pumice stone saturated with sulfuric acid and warmed in a coil of lead tubing submerged in a water bath. The oven was maintained at a temperature of 40° to 43° C. The current of warm dry air was directed against the moist culture so that the evaporation was as great as possible. The culture used in this experiment was 40 gms. of a freshly curdled milk culture of a typical lactic organism to which were added 20 gms. of sterile pulverized lactose.

TABLE 3.

RELATION OF LOSS OF MOISTURE TO DECREASE IN BACTERIA.

| MINUTES FROM BEGINNING OF DRYING | MOISTURE | BACTERIA PER GRAM | |
|---|---|---|---|
| | | Moist Powder | Water-Free Basis |
| | Percentage | | |
| 0 | 59.05 | 785,000,000 | 1,917,000,000 |
| 30 | 48.05 | 750,000,000 | 1,443,000,000 |
| 60 | 34.71 | 963,000,000 | 1,475,000,000 |
| 90 | 24.05 | 942,000,000 | 1,240,000,000 |
| 120 | 10.56 | 916,000,000 | 1,024,000,000 |
| 150 | 4.74 | 351,000,000 | 368,000,000 |
| 1020 | 3.25 | 385,000,000 | 393,000,000 |

In the first 2 hours there was a gradual decrease in the bacteria of nearly 50 per cent, with a drop in the water content to 10.56 per cent. This decrease was fairly uniform in the different periods and may be attributed to the unfavorable temperature conditions to which the culture was exposed.

In the next period of 30 minutes, in which the water content changed from 10.56 per cent to 4.74 per cent, the bacteria dropped from 1,024,000,000 to 368,000,000 per gram. At this time the culture was removed from the drying oven and held until the next morning in a desiccator at room temperature. In this period there was no further decrease in bacteria.

It is evident from this experiment, which was supported by others of a similar nature, that at a water content of between 5 and 10 per cent a concentration is reached which is fatal to a large number of cells. Those that survive become dormant and remain alive for an indefinite period.

LOSS IN RAPID DRYING.

If the assumption be true that exposure to a concentrated medium is responsible in large measure for the death of the bacteria, a method of drying which reduces the time of exposure to this unfavorable condition should diminish the loss of bacteria in the drying process. Fortunately we were able to have a number of cultures dried by a process in which the water was removed from the milk by a spray carried up by a current of warm, dry air. The drying was completed in a very short time, and on account of the rapid evaporation, took place at a low temperature. The cultures dried were of an active lactic acid organism grown on milk. The number of bacteria per gram in various powders made by this method is given in Table 4.

TABLE 4.

BACTERIA IN CULTURES DRIED BY SPRAYING.

| Powder No. | Bacteria per Gram | Powder No. | Bacteria per Gram |
|---|---|---|---|
| 1 | 748,500,000 | 7 | 8,590,000,000 |
| 2 | 1,933,500,000 | 8 | 5,845,000,000 |
| 3 | 4,010,000,000 | 9 | 1,775,000,000 |
| 4 | 6,120,000,000 | 10 | 4,862,000,000 |
| 5 | 1,490,000,000 | 11 | 657,500,000 |
| 6 | 2,015,000,000 | | |

These results show that while there is still a large decrease from the theoretical number present in the milk, the rapid drying gave a powder with a very high bacterial content. These powders gave an active growth when added to milk and produced a good starter on the first inoculation. However, the manufacture of a dry culture by this process requires the use of complicated and expensive machinery and is therefore not within the reach of the ordinary laboratory.

DRYING BY THE FREEZING METHOD.

It is well known that water vapor is given off from ice even when the temperature of the air is below the freezing point of water. If the atmospheric pressure is lowered the rate of evaporation is increased, and if the vapor is removed as fast as it is formed so that the vapor tension is not too high, the entire piece of ice may be evaporated without passing through the fluid state.

This principle has been utilized in a laboratory method of drying first described by Shackell.[1] The method used by him consisted essentially in placing the frozen material in a desiccator over sulfuric acid and exhausting the air either by adding a small amount of ethyl ether and exhausting with a water pump or exhausting the air with a Geryk pump without the aid of ether. The ether vaporizes, displacing the last traces of air, and is itself absorbed by the sulfuric acid. The drying was hastened by rotating the desiccator to replace the saturated layer on the surface with fresh acid. Schackell used this chiefly for moisture determinations but also pointed out its application to other biological problems. Blood was dried without the loss of the dissolved gases or property of clotting and the brain of a rabbit affected with rabies was dried without losing its virulence. Shackell points out that this is probably due to the fact that there is no concentration of the soluble constituents during the drying. The concentration usually incident to drying is avoided because there is no fluid water to hold the solids in solution as the drying progresses.

If the process is interrupted before the drying is complete, it will be found that the outer part of the material will be completely dry, while the inner part is in its original condition. The dry part is porous and friable. Substances which cannot be dried by other means without becoming insoluble in water may by this method be reduced to a powder which is readily soluble in water. The possible value of this method in preparing dry cultures is obvious. Not only is the injurious effect of the increasing concentration during drying removed, but the process is conducted with the cells in a dormant condition.

Hammer[2] compared this method with air drying by moistening strips of filter paper in broth cultures and holding over sulfuric acid in a desiccator submerged in salt and ice and evacuated by a water pump. Cultures dried in this way showed a much greater viability than similar cultures dried over sulfuric acid in an unevacuated desiccator.

Shattock[3] and Dudgeon dried cultures on slips of glass. With some organisms there was little or no difference in the viability of the cultures dried in a vacuum after freezing and those dried in air, but with others, notably *B. pyocyaneus*, the difference in favor of the freezing method was very marked.

The success of this method necessarily depends on the ability of the organism to withstand freezing. It is generally known that many bacteria will survive for long periods in ice, but it is possible that the freezing would destroy a sufficiently large proportion of the bacteria in a culture to affect the activity of the powder.

Macfadyen[4] and Rowland found that *B. acidi lactici*, *B. typhosus*, *B. coli communis*, and many other bacteria as well as a yeast culture survived 10 hours' exposure to a temperature of $-252°$ C. without change in appearance or vitality.

Paul and Prall,[5] who dried staphylococci on garnets and exposed them for one month to the temperature of liquid air, found no diminution in numbers in this period and no change in their resistance to disinfectants. Smith and Swingle[6] found, on the other hand, that when broth cultures are frozen and thawed a large number of the cells succumb and only certain more resistant cells survive. It is possible that the process of concentration taking place as the fluid freezes may have some influence on the

[1] *Am. Jour. Physiol.*, 1909, 24, p. 325.
[2] *Jour. Med. Research*, 1911, N.S., 19, p. 527.
[3] *Proc. Royal Soc.*, 1912, 85, p. 127.
[4] *Ibid.*, 1900, 66, p. 488.
[5] *Arb. a. d. k. Gsndhtsamte.*, 1907, 26, p. 73.
[6] *Science*, 1905, N.S., 21, p. 481.

vitality of the cells. The few determinations that we have made with milk cultures have given no indication of any serious loss due to the freezing. This is seen in Table 5, which shows the effect of freezing on cultures of the lactic bacteria.

These results are substantiated by the activity of the dry cultures made from frozen milk. It is evident that a sufficiently large number, at least, survive the freezing to permit the production of a very active powder.

TABLE 5.

EFFECT OF FREEZING ON BACTERIA IN MILK CULTURES.

| POWDER NO. | BACTERIA PER C.C. | |
|---|---|---|
| | Before Freezing | After Freezing |
| 1 | 2,305,000,000 | 2,290,000,000 |
| 2 | 2,125,000,000 | 1,920,000,000 |
| 3 | 890,000,000 | 1,110,000,000 |
| 4 | 2,300,000,000 | 1,455,000,000 |

METHOD.

In our preliminary work we have used a desiccator for a drying chamber which is very satisfactory for drying small quantities. The culture was usually frozen by flowing in a petri dish and submerging in a mixture of salt and ice. Material may be frozen more quickly and neatly by the use of carbon dioxid snow. We have obtained this snow in the following manner.[1] A one-fourth inch needle valve is connected to the carbon dioxid cylinder by a short piece of pipe, the cylinder inverted, and the valve opened so that the liquid flows into the pipe. This arrangement avoids both the loss due to the cooling of the head of the cylinder as the liquid expands and the plugging of the opening by the formation of ice. A bag made of three or four thicknesses of felt and held in shape by a large cork tacked in one end is held over the end of a pipe a few inches long leading from the needle valve; the valve is then opened, and the snow which forms as the liquid carbon dioxid expands is collected in the bag. More efficient results in freezing may be obtained if the snow is mixed with alcohol, gasoline, or a similar reagent, but for ordinary purposes it is necessary only to place the pan of material to be frozen on a layer of dry snow. The desiccator containing the frozen material was partly covered with salt and ice and the joint between the cover and the desiccator well lubricated with a mixture made by melting together gum rubber and vaseline. Sufficient vaseline must be used to make a soft mixture on the cold glass. When the desiccator is closed the cover should be rotated slightly to eliminate channels through the lubricant. The desiccator is connected with the house vacuum to remove as much air as possible and then with a Geryk pump to secure the high vacuum necessary for rapid drying. The air is passed from the desiccator through a drying train in which it passes in succession over calcium chlorid, sulfuric acid, and phosphorous pentoxid.

[1] The writer is indebted to Mr. F. S. Durston, of the Bureau of Standards, for this suggestion.

We have constructed a drying train from gas-washing towers which has been very satisfactory, not only from the standpoint of efficiency but also on account of the ease with which the drying agents may be replenished. The gas-washing towers are placed in a horizontal position as shown in Fig. 1, and connected with soft rubber tubing which is covered by a mercury seal. The ground joints are well lubricated and rotated carefully to break any channels. It is necessary to make rubber connections between the desiccator and the drying train and between the drying train and the pump. Heavy pressure tubing is used for this purpose. Connected with the drying train is a Plücker tube to indicate the state of the vacuum. It is absolutely essential to the process that a high vacuum be maintained, and to secure this the joints must be perfect and all moisture removed from the air before it passes into the pump.

When a vacuum sufficiently high to produce rapid drying has been secured, the Plücker tube will show a fluorescence indicating a vacuum of about 0.01 mm. The time required to produce this will depend, of course, on the efficiency of the pump and the drying train and the capacity of the desiccator. With our apparatus, using one desiccator, it usually required less than half an hour.

The time required for complete drying varies with the relative amount of material and sulfuric acid, the relative amount of surface exposed in the material, and the completeness of the vacuum. We usually dried in a desiccator about 10 c.c. of milk culture. Under ordinary circumstances this would be completely dried in 3 or 4 hours, but we have made a practice of disconnecting the desiccator when the necessary vacuum is secured and holding overnight in a refrigerator maintained at a temperature a few degrees below freezing.

## COLLECTING THE MOISTURE.

It is very essential to the success of the process that an efficient agent be provided to absorb the moisture as rapidly as it is given off by the material undergoing desiccation. The difference in vapor tension between the material and the absorbent must be great in order to insure a rapid transfer of moisture from one to the other. Phosphorus pentoxid answers this requirement, but on account of its cost and the nature of this reagent which makes it disagreeable to handle it is not well adapted for use on an extensive scale. Concentrated sulfuric acid which has a vapor tension near zero is nearly as efficient and has the advanatge of being available for repeated use by reconcentrating. Lime, which has the advantage of cheapness and of presenting a very large surface, has not been found satisfactory. Its action is too slow to make it a possible substitute for sulfuric acid.

Dr. Frankland of the Public Health Service has suggested the possibility of condensing moisture in a secondary chamber held at a very low temperature and has successfully used liquid air for this

purpose. The vapor tension of ice at 0° C. is 4.579, while at −50° C., the lowest temperature given in Landolt-Börnstein's tables, the vapor tension is 0.034. At the temperature of liquid air, which is about −175° C., the vapor tension would be very low indeed, and there would be a rapid transfer of moisture from the ice at 0° to that at −175°. Carbon dioxid gas, which is the reagent used in many refrigerating machines, has a boiling point of −78° C. Ammonia, which is more commonly used in this country, gives, with a back pressure of 10 pounds, a temperature of about −35° C., which corresponds to a vapor tension of 0.173.

One experiment was made using carbon dioxid snow to cool a condenser connected with a desiccator. A small desiccator was connected by a one-half-inch tube with a round-bottomed flask of 500 c.c. capacity. The tube was cemented to the desiccator and the flask. In the desiccator was placed a crystallizing dish containing 10 gms. of ice. The desiccator was packed in ice mixed with a small amount of salt. When the system was evacuated to about 0.01 mm. the flask was packed in carbon dioxid snow and held in this way for 4 hours. At the end of this time it was found that the ice had lost only about 0.3 gm. While the difference in vapor tension between the ice in the desiccator and that on the inner surface of the flask which served as a condenser was great, it evidently was not sufficient to cause a rapid transfer of moisture from one to the other.

### DRYING IN LARGE QUANTITIES.

In order to secure larger quantities of powder a large drying chamber was constructed, using a 10-inch cast-iron pipe with one end closed by a stock cap. On the top was threaded a standard cast-iron flange fitted with a heavy cast-iron cover. The cover and the flange were ground true and a tight joint made by a rubber gasket. It was necessary, however, to clamp the cover down to make a perfectly tight joint. Some difficulty was encountered at first on account of a pinhole through the cast-iron cover, but this was overcome by painting the cover with shellac.

The drying chamber was held in an insulated tank so constructed that salt and ice could be packed about it in sufficient quantities to hold the temperature below freezing for several hours. A rack supporting 10 circular shelves was used to hold 4 lead pans for sulfuric acid and 6 tin trays for the culture. The cultures were frozen in the trays by packing in salt and ice, the covers removed and the trays placed on the shelves, alternating with the pans of acid. The rack was placed in the drying chamber, the cover clamped in place, and water added to the ice and salt mixture in the tank until the joint between the cover and the flange was covered. It is very difficult to make a rubber-joint vacuum tight, but by covering the rubber with a fluid this can usually be accomplished. As much as possible of the air was then removed by a connection with the house vacuum and the residue exhausted with the Geryk pump. With our apparatus this usually required 3 or 4 hours.

To avoid the necessity of submerging the tank in salt and ice a new apparatus was constructed as shown diagrammatically in Fig. 1. In place of the tank the drying

chamber is held in a horizontal position in a refrigerator maintained at a temperature below freezing by brine coils. The flange is grooved to hold two gaskets as shown in Fig. 2, thus providing a space which can be filled by means of a rubber tube connected at the lower opening, *A*, with brine, oil, or mercury. The vent, *B*, permits the escape

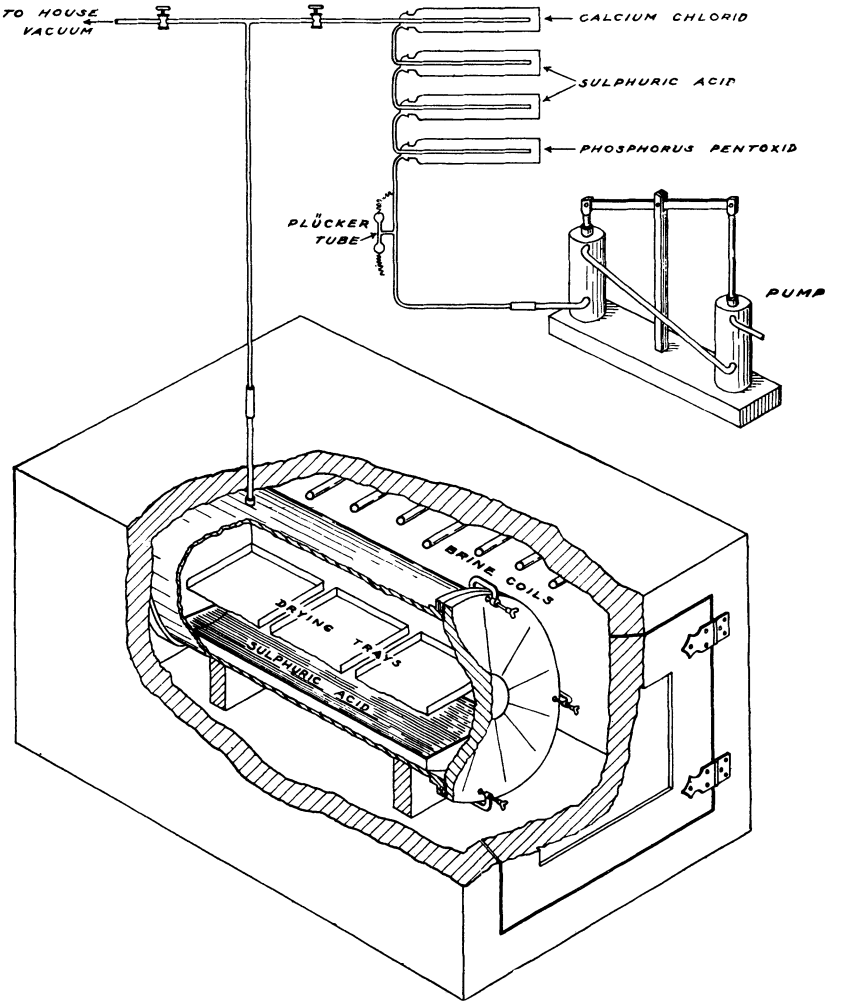


FIG. 1.—Apparatus for drying large quantities of culture.

of the air as the fluid fills the annular space between the gaskets. In this way a vacuum-tight joint may be obtained without excessive pressure on the cover. Similar results may be obtained by using a lead gasket, but this requires a high pressure on the cover to insure a tight joint.

### DRYING LACTIC CULTURES.

In drying by this process a freshly curdled milk culture was used. This may be ordinary sterile milk inoculated and incubated overnight at 30° C., or, for reasons which will be pointed out later, cultures may be made on milk concentrated to one-half to one-fourth of its original volume. The culture, which is frozen hard, comes from the drier in a very friable condition and is easily reduced to a powder. Powders made from concentrated milk contain so much sugar that they become slightly plastic shortly after they are taken from the drier. However, this does not prevent their being ground in a mortar.

Table 6 shows the number of bacteria per gram in various powders made from cultures suitable for cream ripening.

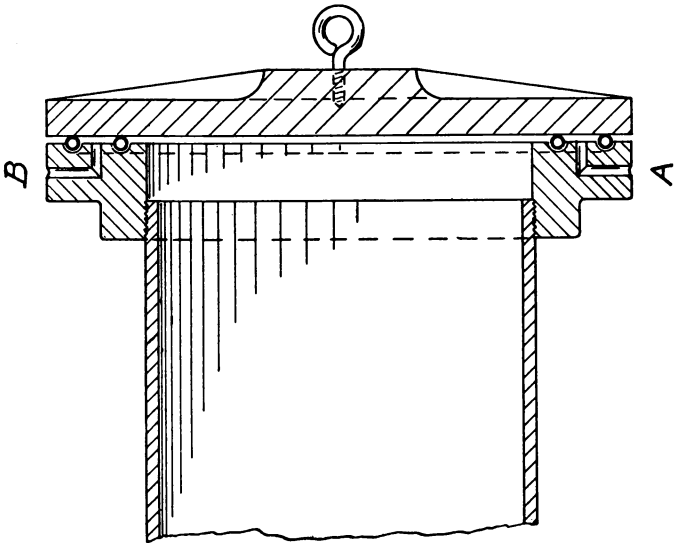


FIG. 2.—Arrangement for obtaining a vacuum-tight joint.

It is difficult to get an accurate count on these powders because the particles of acid curd are not readily soluble in the dilution water and undoubtedly many bacteria are held in these particles. Notwithstanding this fact Table 6 shows that a powder containing a large number of bacteria can be made by this process.

TABLE 6.

BACTERIA IN LACTIC CULTURES DRIED BY FREEZING METHOD.

| Powder No. | Bacteria per Gram | Powder No. | Bacteria per Gram |
|---|---|---|---|
| 1 | 8,030,000,000 | 7 | 380,000,000 |
| 2 | 7,737,000,000 | 8 | 4,900,000,000 |
| 3 | 12,670,000,000 | 9 | 724,000,000 |
| 4 | 6,770,000,000 | 10 | 929,000,000 |
| 5 | 10,180,000,000 | 11 | 1,020,000,000 |
| 6 | 385,000,000 | | |

The number of bacteria found by the plating method is not always a good indication of the activity of the powder as an agent for souring milk, and another method has been used to test this point. One milligram weighed on a chemical balance is added to 1,000 c.c. of sterile milk and incubated at 30° C. The activity of the powder is indicated by the increase in acidity of the milk, which is titrated at stated intervals. On account of the difficulty of weighing out this small amount of powder with even reasonable exactness the procedure was varied by weighing one-half a gram into a water blank from which a definite dilution may be made. The probable accuracy of this method is shown in Table 7, which gives the results obtained by making six separate inoculations from one lot of powder in 1000 c.c. flasks of milk.

In three of the flasks the powder was added in the ratio of 1 to 1,000,000 and in the others in a ratio of 1 to 40,000,000. It is evident that while this method gives a good indication of the activity of the powder, it can be depended upon for minor differences only when similar results are obtained on repeated trials.

The activity of a number of powders tested in this way may be seen in Table 9, on p. 113. The milks were inoculated with powder at the rate of 1 part of powder in 40,000,000 parts of milk.

TABLE 7.

METHOD OF TESTING CULTURES.

| DILUTION OF POWDER IN MILK | ACIDITY OF MILK AFTER | |
|---|---|---|
| | 18 Hrs. | 20 Hrs. |
| | Percentage | Percentage |
| 1:1,000,000 | .594 | .720 |
| | .729 | .783 |
| | .684 | .756 |
| 1:40,000,000 | .207 | .315 |
| | .189 | .261 |
| | .198 | .306 |

These results should be compared with those given in Table 2, which shows the acidity developed by commercial cultures under similar conditions except that the dilution used was very much less. The cultures dried by freezing gave a clean-flavored curd on the first inoculation without evidences of contamination.

### INCREASING THE NUMBER OF BACTERIA BY NEUTRALIZATION.

In order to secure a powder with the required number of active bacteria it is necessary to start with a culture containing a large number of growing cells. A freshly curdled milk culture of an ordinary lactic bacterium will usually contain, by the accepted methods of counting, 1 to 3 billions per cubic centimeter. This will depend somewhat on the activity of the culture, and some strains may cease multiplication before this number is reached. It is well known that the growth period may be extended by various methods of neutralization and this has been made use of in the addition of phosphates to sugar broths. An attempt was made to increase the number of bacteria in milk cultures before drying by this means. Sterile milk containing 1 per cent dibasic potassium phosphate was inoculated with a freshly curdled

milk culture and held with suitable check without potassium phosphate at 30° C. The results are given in Table 8.

TABLE 8.

INFLUENCE OF POTASSIUM PHOSPHATE ON GROWTH OF BACTERIA.

| Lot No. | Age | Acidity | | Bacteria (Millions per c.c.) | |
|---|---|---|---|---|---|
| | | Normal | Potassium Phosphate | Normal | Potassium Phosphate |
| | Hrs. | Percentage | Percentage | | |
| 1 | 0 | .216 | .297 | 7 | 8 |
| | 12 | .801 | 1.059 | 2,590 | 536 |
| | 15 | .882 | 1.332 | 3,640 | 5,020 |
| | 19 | .918 | 1.449 | 2,690 | 4,830 |
| | 36 | 1.071 | 1.615 | 2,890 | 18,880 |
| 2 | 0 | ..... | ..... | 7 | 6 |
| | 12 | .669 | .779 | 2,630 | 1,400 |
| | 15 | .801 | .801 | 2,340 | 2,850 |
| | 19 | .826 | .829 | 2,290 | 2,100 |
| | 40 | .855 | .963 | 2,550 | 1,730 |
| 3 | 0 | .243 | .237 | 9 | 8 |
| | 12 | .871 | 1.180 | 1,620 | 2,960 |
| | 15 | .918 | 1.198 | 1,690 | 2,570 |
| | 19 | .972 | 1.198 | 2,130 | 2,060 |
| | 36 | .972 | 1.242 | 2,770 | 3,820 |
| 4 | 0 | .297 | .297 | 4 | 4 |
| | 12 | .756 | .855 | 2,530 | 3,160 |
| | 15 | .880 | 1.282 | 2,210 | 4,150 |
| | 19 | .900 | 1.395 | ..... | ..... |
| | 36 | .999 | 1.638 | 2,510 | 4,340 |
| 5 | 0 | .200 | .255 | 8 | 8 |
| | 12 | .715 | .994 | 2,420 | 3,470 |
| | 15 | .846 | 1.166 | 2,780 | 3,650 |
| | 18 | .846 | 1.224 | 2,540 | 4,260 |
| | 36 | .972 | 1.368 | 2,360 | 3,990 |
| 6 | 0 | .155 | .216 | 9 | 10 |
| | 2 | .171 | .243 | 111 | 93 |
| | 4 | .186 | .257 | 220 | 219 |
| | 6 | .252 | .576 | 650 | 700 |
| | 10 | .720 | .860 | 2,580 | 3,300 |
| | 24 | .949 | 1.521 | 2,520 | 5,070 |
| | 30 | .936 | 1.476 | 2,770 | 4,680 |
| 7 | 0 | .167 | .239 | 9 | 7 |
| | 2 | .180 | .254 | 14 | 10 |
| | 4 | .196 | .266 | 80 | 69 |
| | 7 | .257 | .326 | 743 | 169 |
| | 11 | .703 | .745 | 2,320 | 2,720 |
| | 24 | .967 | 1.363 | 3,310 | 3,550 |
| | 31 | .999 | 1.404 | 5,070 | 4,770 |
| 8 | 0 | .167 | .239 | 8 | 6 |
| | 2 | .180 | .248 | 6 | 15 |
| | 4 | .194 | .252 | 9 | 59 |
| | 7 | .247 | .279 | 230 | 327 |
| | 11 | .531 | .540 | 322 | 2,240 |
| | 24 | .837 | 1.359 | 2,480 | 4,790 |
| | 31 | .909 | 1.422 | 1,480 | 3,940 |

The number of bacteria in milk in which the acidity was neutralized by potassium phosphate was usually considerably greater than in the corresponding unneutralized culture, but when these milks were dried by the spraying method the unneutralized milks gave the more active powder. Evidently the acid phosphate had a detrimental effect when it was concentrated.

Somewhat similar results were obtained in cultures made by the freezing method from lactic milk cultures one-half of which were neutralized by the addition of 2 per cent calcium carbonate. The results, which are given in Table 9, are contradictory and show no special advantage in neutralizing before drying.

TABLE 9.

EFFECT OF NEUTRALIZING MILK BEFORE DRYING ON ACTIVITY OF CULTURE.

| POWDER NO. | TIME FROM INOCULATION | ACIDITY OF MILK INOCULATED WITH CULTURE FROM | |
|---|---|---|---|
| | | Unneutralized Milk | 2 Per Cent $CaCO_3$ |
| | Hrs. | Percentage | Percentage |
| 1 | 17 | .194 | .347 |
| | 19 | .288 | .661 |
| | 21 | .626 | .779 |
| 2 | 17 | .200 | .270 |
| | 19 | .297 | .594 |
| | 21 | .513 | .675 |
| 3 | 17 | .153 | .153 |
| | 22 | .243 | .207 |
| | 24 | .567 | .360 |
| 4 | 17 | .702 | .702 |
| | 19 | .837 | .837 |
| 5 | 17 | .414 | .189 |
| | 23 | .738 | .468 |
| | 25 | .738 | .675 |
| 6 | 18 | .738 | .693 |
| | 20 | .792 | .738 |
| 7 | 17 | .216 | .247 |
| | 19 | .342 | .427 |
| | 21 | .666 | .693 |
| 8 | 17 | .171 | .185 |
| | 19 | .211 | .216 |
| | 21 | .333 | .302 |
| | 23 | .598 | .545 |
| 9 | 18 | .288 | .216 |
| | 20 | .468 | .378 |

By neutralizing *B. bulgaricus* cultures at different stages of growth somewhat similar results were obtained. Cultures in milk concentrated to one-half its volume were grown 24 hours at 37° C. At the end of that time a portion was dried and the remainder partially neutralized and again incubated. This could not be done accurately on account of the high percentage of casein. After 48 hours a second portion was dried and the remainder neutralized, and at 72 hours the third portion was dried. The three lots of powder thus obtained were tested by adding 10 milligrams to 500 c.c. of milk which was incubated at 37° C. and the acidity titrated at regular intervals. The results, which are given in Table 10, show that the most active powders are obtained from the unneutralized milk.

The neutralization evidently did not stimulate the growth of this organism. On the contrary the milk of Portion 5 contained at 24 hours 12,400,000 per cubic centimeter, at 48 hours 3,500,000 per cubic centimeter, and at 72 hours only 100,000 per cubic centimeter.

BY CONCENTRATION OF THE MILK.

The observation was made by Mr. Ayres of this laboratory in the course of another investigation that *B. bulgaricus* gave a more vigorous growth in a concentrated milk or whey. It was thought that similar methods with the ordinary lactic type might produce a more active powder. To test this a quantity of milk, concentrated in a vacuum to one-half its volume and sterilized in an autoclav, was inoculated with a good lactic culture and incubated at 30° C. for about 18 hours. Similar cultures were made in normal sterile milk and incubated with the concentrated-milk culture. When these were dried by the freezing method and compared by inoculating a definite amount into milk, as shown in Table 11, there was no great difference in the activity of the two powders.

TABLE 10.

INFLUENCE OF NEUTRALIZATION ON ACTIVITY OF BULGARICUS POWDERS.

| POWDER NO. | 24-HOUR CULTURE NOT NEUTRALIZED | | | | 48-HOUR CULTURE NEUTRALIZED AT 24 HOURS | | | | 72-HOUR CULTURE NEUTRALIZED AT 24 AND 48 HOURS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Acidity of Milk Inoculated with Powder after | | | | Acidity of Milk Inoculated with Powder after | | | | Acidity of Milk Inoculated with Powder after | | | |
| | 17 Hrs. | 19 Hrs. | 21 Hrs. | 23 Hrs. | 17 Hrs. | 19 Hrs. | 22 Hrs. | 23 Hrs. | 17 Hrs. | 19 Hrs. | 21 Hrs. | 23 Hrs. |
| | % | % | % | % | % | % | % | % | % | % | % | % |
| 1 | .261 | .396 | .594 | .738 | .177 | .180 | .207 | .216 | ..... | ..... | ..... | ..... |
| 2 | .279 | .333 | .387 | .621 | .135 | .144 | .153 | .171 | .144 | .153 | .153 | .162 |
| 3 | .693 | .711 | .720 | .738 | .232 | .288 | .387 | .576 | .189 | .261 | .342 | .423 |
| 4 | .180 | .180 | .198 | .243 | .162 | .198 | .288 | .630 | .153 | .180 | .243 | .441 |
| 5 | ..... | .450 | .522 | .666 | ..... | .333 | .432 | .603 | ..... | .279 | .297 | .360 |

TABLE 11.

RELATIVE ACTIVITY OF POWDERS FROM PLAIN AND CONCENTRATED MILK.

| POWDER NO. | DILUTION OF POWDER | POWDER FROM PLAIN MILK | | | POWDER FROM CONCENTRATED MILK | | |
|---|---|---|---|---|---|---|---|
| | | 17 Hrs. | 19 Hrs. | 21 Hrs. | 17 Hrs. | 19 Hrs. | 21 Hrs. |
| | | Percentage | Percentage | Percentage | Percentage | Percentage | Percentage |
| 1 | 1: 1,000,000 | .702 | .837 | ..... | .531 | .774 | ..... |
| 2 | 1: 1,000,000 | .738 | .792 | ..... | .666 | .738 | ..... |
| 3 | 1:40,000,000 | .216 | .342 | .666 | .207 | .333 | .616 |

There is, however, a decided advantage in the use of the concentrated milk. Weight for weight, the powder is as active as that obtained by drying plain milk, and since about half of the water has been removed before drying, twice as much powder can be produced in each operation of the drier when a culture grown on concentrated milk is used. For laboratory purposes concentrated milk can be easily produced by boiling skim milk in a large round-bottomed flask connected by a condenser with a large vacuum flask.

If a vacuum of 26 inches or more is maintained, rapid evaporation takes place at a low temperature. Milk may be concentrated in this way to one-fourth its original volume, but on sterilizing this forms a thick coagulum that is difficult to handle, so that milk concentrated to one-half its volume is more satisfactory.

### DRYING *B. Bulgaricus.*

The great demand for cultures of this group to use in fermenting milk or for direct consumption for therapeutic purposes has given special interest to attempts to produce an active powder by the freezing method. The commercial powders made in various ways have not proved satisfactory, due in part to the crude methods of drying and partly to the fact that the decrease in activity after drying is rapid.

Our results have shown that it is possible to make a dry culture of *B. bulgaricus* containing over 1,000,000,000 cells per gm. These powders, made by the freezing method, when added to milk in the ratio of 1 part of powder to 1,000,000 parts of milk and incubated at 37° C., give an acidity of from 0.6 to 0.8 per cent in 24 hours and a curd free from any evidences of contamination. The best powder is made from a culture grown on concentrated milk 48 hours at 37° C.

In using these organisms for therapeutic purposes there are certain advantages in an active powder, in addition to its use in preparing sour milk. The culture is used to introduce into the digestive tract as many acid-forming organisms as possible, and for this purpose probably nothing is better than a good milk culture. But in many cases the patient objects to the taste of the milk, or it is inadvisable to introduce into the diet so much nitrogenous material. There is also the difficulty of obtaining or preparing the milk culture. Under these conditions a dry powder containing a large number of active cells would be of great advantage.

### DRYING LABORATORY CULTURES.

The difficulties in carrying stock cultures are too well recognized to need discussion. Frequent transfers are necessary to insure the maintenance of many cultures, there is always the danger of contamination by molds, and the salient characters of some cultures are slowly changed by continued growth on artificial media. Every bacteriologist has been confronted with the question of discarding cultures which may at some indefinite future time be of great value or of continuing their transfer at considerable trouble and with the knowledge that many of them will eventually be lost. The advantage of having a culture in such a condition that it can be put away in the refrigerator and held for months or years with its characters unimpaired is obvious.

We have dried a large number of cultures of various kinds, and altho the time since drying has not yet been long enough to warrant definite conclusions, it is evident that the cultures may be maintained in the dry condition for many months, especially if they are held in a refrigerator. These cultures were dried at first in small phials plugged with cotton. It was found, however, that ordinary test tubes were more satisfactory for this purpose. The tube should be of a large diameter, and the plug should be loose to permit a free flow of air and moisture out of the tube. If too much of the medium is used it will not become dry in a reasonable time. We have found that when drying milk cultures 3 or 4 c.c. is sufficient. This dries quickly and gives an abundance of powder.

We have dried in this way many lactic-acid cultures, cultures occurring in Swiss cheese, and cultures of the *coli* group. Much trouble has been experienced from the difficulty in keeping cultures of the latter group in a frozen condition, due, no doubt, to the amount of alcohol and other volatile products formed by the fermentation of the sugar. This difficulty has been obviated by growing them in a medium made by adding 2 or 3 per cent of casein to sugar-free broth. Sufficient growth is obtained without the formation of alcohol and the casein acts as a filler for the powder.

It is apparent, however, that the ability to withstand this process varies with different organisms. It is also possible that varying results may be due to the inability of some organisms to give a luxuriant growth in the medium used. Whatever the cause may be, the number of failures to revive after long periods has been great enough to preclude an unconditional recommendation for this purpose.

### DRYING CULTURES OF THE LEGUME BACTERIA.

Within recent years the advantage of inoculating certain soils has been abundantly demonstrated, and various methods have been devised for distributing cultures for this purpose. None of the dried cultures have proved to be entirely satisfactory, and the legume bacteria are disseminated by fluid or semi-fluid cultures or by the transfer of infected soil.

A culture of this type isolated from the nodules of sweet clover and grown in milk was dried by the freezing method and a powder obtained containing in each gram about 1,000,000,000 cells. After 6 months at room temperature the powder gave typical growth in a dilution of 1 to 1,000,000, but no growth in the 1 to 10,000,000 dilution.

Since this organism does not ferment lactose, no appreciable acidity is formed in the milk and the powder obtained dissolves quickly and completely in water.

### DRYING YEASTS.

It is probable that yeast cells are too large to be dried by any method requiring freezing. Cultures of a bread-yeast fermenting saccharose but not lactose have been dried, but the powder when reinoculated into milk containing saccharose gave evidence of gas formation only after several days' incubation. Similar results were obtained with a powder made by freezing and drying the sediment obtained by centrifuging a dextrose broth culture of the bread yeast. The powder obtained in this way was composed almost entirely of yeast cells which were, so far as microscopic examinations showed, intact, but gave no fermentation when inoculated into sugar broth.

## CONDITIONS INFLUENCING LOSS OF VITALITY.

The question of retention of vitality is of scientific as well as practical importance. Any culture gradually loses its vitality until it can no longer be reproduced. The rate of this loss is dependent on several conditions, all of which have an influence on the functions which maintain the vital activities of the organism. A culture grows more rapidly, reproduces itself more quickly, and dies earlier at its optimum temperature than at temperatures near the lower thermal limits of its activity. The same relations hold at temperatures too low to permit actual growth. An organism whose normal life cycle occupies a few days may live for months in a dormant condition, but even this existence is limited. A seed that will germinate in a few days in a moist condition may retain the power of

germination for months or even years if the moisture is withheld. The nearer the cell approaches an absolutely dormant condition the longer its actual death will be postponed.

The dormancy is necessarily only relative, as it is hardly possible that all action is suspended, even under conditions of extreme dryness or low temperature. The fact that life finally ceases even under these conditions of apparently complete suspension in itself proves that there are still changes taking place in the protoplasm, since the transition from the state in which it is capable of reproducing itself to the state in which this power is lost presupposes some chemical activity. The main consideration in preserving a culture is to attain a condition of dormancy as complete as possible, in order that the vital activities may be reduced to the lowest possible ebb without completely extinguishing them.

Four of the elementary necessities for maintaining life are moisture, heat, oxygen, and food. Obviously the latter becomes unavailable when moisture is withdrawn. The length of time a dry culture retains its vitality may be influenced by the degree of its dryness (in other words, the amount of moisture it contains), the temperature at which it is held, and possibly by the nature of the gas in which it is held.

### INFLUENCE OF MOISTURE.

It is to be expected that the time a powder would retain its activity would be influenced by the amount of moisture it contained, since the life of the cell is dependent on a supply of water and its metabolic activity ceases when water is withdrawn. This was found to be true in an experiment conducted in the following manner:

A portion of a powder of fair activity was exposed overnight in a desiccator over sulfuric acid; another portion was exposed in a similar way for 3 hours over water. The original powder was found to contain 1.39 per cent of moisture, that exposed to sulfuric acid 0.90 per cent, and that held over water 5.77 per cent. The 3 lots were sealed in small phials and held in an incubator at a temperature of 28° C. At the end of 157 days 1 gm. of each lot was weighed with proper corrections for moisture content into flasks each containing 200 c.c. of milk, these were warmed to 30° C. and incubated at 30° C. overnight. The relative activity of the powders was measured by the titration of the milk at stated intervals. The results, given in Table 12, are averages of 2 flasks made on different days.

It will be noted that while there was no appreciable difference in the results with the powders containing 0.90 and 1.39 per cent of moisture, the powder with 5.77 per cent had fallen off distinctly in its activity.

To obtain the best results cultures should be dried as completely as possible and placed at once in a package that will exclude the possibility of their absorbing moisture from damp air.

TABLE 12.

INFLUENCE OF MOISTURE CONTENT ON LOSS OF VITALITY.

| HOURS FROM INOCULATION | ACIDITY IN MILK | | |
|---|---|---|---|
| | 0.90 Per Cent Moisture | 1.39 Per Cent Moisture | 5.77 Per Cent Moisture |
| | Percentage | Percentage | Percentage |
| 0 | .171 | .171 | .171 |
| 17:30 | .279 | .243 | .234 |
| 19:30 | .315 | .351 | .261 |
| 21:30 | .342 | .405 | .297 |
| 23:30 | .459 | .549 | .342 |

## INFLUENCE OF TEMPERATURE.

It is almost an axiom that bacteria succumb to unfavorable conditions most quickly at temperatures at which they grow most rapidly. A lactic culture survives for a long time in a refrigerator but lives only a few days in an incubator. Accordingly we should expect that dried cultures would deteriorate more rapidly in a warm room than in a refrigerator.

Paul[1] dried staphylococci on garnets and exposed them at room temperature, in an icebox, and in liquid air. At the end of a 32-day-period the bacteria at room temperature had decreased from 90,800 to 300. At the icebox temperature the decrease was less rapid but was marked, while at the temperature of liquid air there was no appreciable change.

A number of tests were made to determine the relative rate of deterioration at different temperatures by holding parts of the same powder at the various constant temperatures available in the laboratory and, after long periods, determining the activity of the powder by adding a definite amount to milk and measuring the rate of acid formation. One milligram of the powder was weighed out and added to 1,000 c.c. of milk which was incubated at 30° C. and the activity titrated at the end of 17, 19, and 21 hours. The results of one of these, which is representative, are given in Table 13.

In 30 days a distinct loss in activity had taken place at the higher temperatures. In 60 days at 30° C. and 37° C. the bacteria were nearly or quite dead, and at 18° C. the deterioration was marked. At 0° and −6° C. there was some loss, but the powder was still very active.

Table 14 shows the results of another test in which the bacteria were determined in a powder after 128 days at various temperatures. The powder which was dried in a current of air, contained originally a little over 3,000,000 bacteria per gram. At 0° C. the loss was within the limits of error, but at 5° to 10° C. and at 17° C. there was a 30 per cent loss, while at 30° C. the decrease exceeded 60 per cent.

[1] *Biochem. Ztschr.*, 1909, 18, p. 1.

### INFLUENCE OF ATMOSPHERE IN WHICH CULTURE IS HELD.

It is reasonable to suppose that dried cultures would be affected to some extent by the gas in which they are held. Many bacteria are more active in the presence of oxygen than in its absence. Marshall[1] has observed that carbon dioxid has a detrimental influence on some bacteria.

The relative rate of loss of vitality as influenced by different gases was determined by holding about 1 gm. of powder in tubes in which the air was displaced by the gases studied. One tube was sealed without displacing the air, and in one tube in each set the air was exhausted by a Geryk pump giving a vacuum of about 0.01 mm. In other tubes the air was displaced by oxygen, hydrogen, nitrogen, and carbon dioxid. The oxygen was obtained by dropping boiled distiller water slowly on solid sodium peroxid and washing the gas with water and drying with sulfuric acid.

TABLE 13.

INFLUENCE OF TEMPERATURE OF STORAGE ON LOSS OF VITALITY.

| HOURS FROM INOCULATION | ACIDITY AFTER 30 DAYS AT | | | | | | ACIDITY AFTER 60 DAYS AT | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh Culture | −6° C. | 0° C. | 18° C. | 30° C. | 37° C. | −6° C. | 0° C. | 18° C. | 30° C. | 37° C. |
| | Per-centage | Per-centage | Per-centage | Per-centage | Per-centage | Per-centage | Per-centage | Per-centage | Per-centage | Per-centage | Per-centage |
| 17 | .362 | .369 | .329 | .257 | .156 | .149 | .288 | .274 | .193 | .180 | .198 |
| 19 | .495 | .689 | .779 | .752 | .424 | .158 | .355 | .369 | .193 | .175 | .193 |
| 21 | .657 | ..... | ..... | ..... | ..... | ..... | .423 | .553 | .229 | .175 | .180 |

TABLE 14.

INFLUENCE OF TEMPERATURE OF STORAGE ON LOSS OF VITALITY.

| AGE OF POWDER | BACTERIA PER GRAM IN POWDER HELD AT | | | |
|---|---|---|---|---|
| | 0° C. | 5–10° C. | 17° C. | 30° C. |
| Initial | 3,270,000 | 3,270,000 | 3,270,000 | 3,270,000 |
| 128 days | 3,180,000 | 2,420,000 | 2,500,000 | 930,000 |

Hydrogen was obtained by running dilute hydrochloric acid on chemically pure zinc. The gas was purified by passing through a solution of lead acetate and over broken earthenware covered with a paste of silver sulfate and dried by passing over phosphorous pentoxid.

Nitrogen was obtained by drawing air (1) through concentrated sulfuric acid, (2) through alkalin pyrogallol, (3) over heated copper oxid, (4) over heated copper spiral, (5) through potassium hydrate, and (6) over calcium chlorid.

The carbon dioxid was made by dropping dilute sulfuric acid slowly on sodium carbonate, and purified by passing through water and sulfuric acid. The tubes in which the powder was held were made with a bulb in the middle to hold the powder and capillary tubes at each end one of which was necessarily sealed on after the tube

[1] *Centralbl. f. Bakteriol.*, 1902, 9, pp. 313, 372, 429, and 483.

was filled. The gas was passed through for 1 to 2 hours and the capillary tubes were sealed while the gas was still flowing.

Several sets of tubes were held in this way under varying conditions. The results of two of these experiments are given in Table 15, which shows the number of bacteria in each gram of the powder before and after the exposure to the different gases, and Table 16, in which are tabulated the results obtained from another lot by inoculating the powders into milk.

TABLE 15.

INFLUENCE OF VARIOUS GASES ON LOSS OF VITALITY.

| HELD IN ATMOSPHERE OF | BACTERIA PER GRAM | |
|---|---|---|
| | Initial | After 132 Days |
| Air | 3,270,000 | 750 |
| Oxygen | 3,270,000 | 7,750 |
| Hydrogen | 3,270,000 | 350,500 |
| Nitrogen | 3,270,000 | 22,000 |
| Carbon dioxid | 3,270,000 | 276,500 |
| Vacuum | 3,270,000 | 785,000 |

TABLE 16.

VIABILITY OF DRIED CULTURES HELD IN VARIOUS GASES.

Acidity of Milk Inoculated with Cultures after 30 Days' Exposure at 30° C., One Part Powder to 40,000,000 Parts Milk.

| TIME FROM INOCULATION | ACIDITY OF MILK INOCULATED WITH CULTURE HELD IN | | | | | |
|---|---|---|---|---|---|---|
| | Air | Oxygen | Nitrogen | Hydrogen | Carbon dioxid | Vacuum |
| | Percentage | Percentage | Percentage | Percentage | Percentage | Percentage |
| 15 hours | .175 | .162 | .189 | .207 | .162 | .180 |
| 19 hours | .166 | .162 | .175 | .283 | .162 | .202 |
| 21 hours | .162 | .189 | .198 | .450 | .166 | .301 |
| 23 hours | ..... | .261 | .360 | .589 | .175 | .625 |
| SAME POWDER AFTER 45 DAYS AT 30° C. ONE PART POWDER TO 1,000,000 PARTS MILK. | | | | | | |
| 18 hours | .171 | .144 | .144 | .171 | .144 | .252 |
| 20 hours | .171 | .144 | .144 | .171 | .144 | .549 |
| 24 hours | .171 | .144 | .189 | .396 | .162 | .702 |

There are some discrepancies in these results as to the relative loss of vitality in different gases, but they all agree in showing the greatest losses in air and in oxygen and the greatest activity in the powder held in the vacuum. The activity of the powder held in the inert gases, hydrogen and nitrogen, was usually greater than that held in carbon dioxid, and always decidedly greater than that held in air or oxygen, but less than in the vacuum tube. It will be noticed that Table 15 shows more bacteria in the powder held in

TABLE 17.

COMPARATIVE VIABILITY OF BULGARICUS POWDERS IN AIR AND IN VACUUM.

| AGE OF CULTURE | HELD IN AIR | | | | | HELD IN VACUUM | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hours | | | | | Hours | | | | |
| | 17 | 19 | 21 | 23 | 25 | 17 | 19 | 21 | 23 | 25 |
| Days | Percentage of Acidity | | | | | Percentage of Acidity | | | | |
| 0..... | .225 | .351 | .441 | .486 | ..... | .225 | .351 | .441 | .486 | ..... |
| 7..... | .189 | .288 | .342 | .504 | ..... | .189 | .270 | .351 | .540 | ..... |
| 14..... | .162 | .189 | .234 | .270 | ..... | .189 | .207 | .243 | .324 | ..... |
| 21..... | .144 | .189 | .279 | .396 | ..... | .171 | .243 | .351 | .513 | ..... |
| 28..... | .189 | .216 | .288 | .342 | ..... | .207 | .297 | .414 | .603 | ..... |
| 42..... | .171 | ..... | .234 | .333 | ..... | .216 | ..... | .387 | .621 | ..... |
| 101..... | .171 | .171 | ..... | .171 | .216 | .216 | .288 | ..... | .342 | .486 |

carbon dioxid than in that held in nitrogen. In all other cases, however, the powder held in carbon dioxid was weaker than those exposed to nitrogen or hydrogen. It is difficult to explain the greater decrease in activity in the tubes filled with air than that in tubes filled with the individual gases occurring in air. It is possible that the air, which was inclosed without drying, may have held more moisture than the other gases, which were carefully dried, and this additional moisture may account for the difference in the effect on the powders.

*B. bulgaricus* cultures held in a vacuum show an activity distinctly greater than parts of the same powder held in air. This is illustrated by the results shown in Table 17, which were obtained by sealing portions of a *B. bulgaricus* powder in small phials and holding at room temperature with similar portions from the same powder which were held in sealed evacuated tubes. A definite amount of the powder was added to one liter of milk which was incubated at 37° C. and the acidity determined at 17, 19, 21, and 23-hour periods.

In 42 days the cultures in air were perceptibly weakened while those in vacuum were apparently

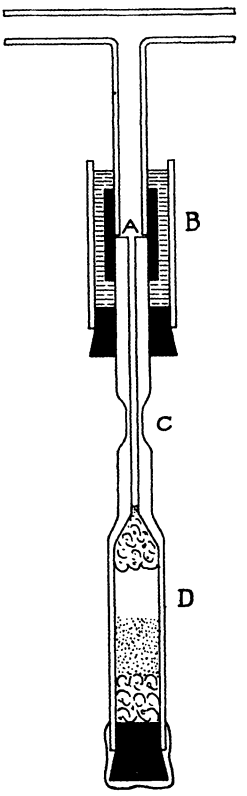


FIG. 3.—Apparatus for evacuating small tubes of powder.

unchanged. This observation is utilized in preparing cultures for distribution. For this purpose tubes are made as shown in Fig. 3. The powder is placed in Tube *D* between cotton plugs, a stopper inserted and covered with a cement made by boiling together resin, gum rubber, and a little vaseline. Connection between these tubes and the pump is made by means of a glass tube with a number of side arms. The joint at *A* is made by a rubber tube, inclosed in a glass tube, *B*, held in place by a rubber stopper. This tube is filled with mercury so that the rubber tube is covered. After the evacuation is complete the tube is sealed off at the constriction, *C*.

## SUMMARY.

Commercial dry cultures of the lactic acid bacteria usually contain a small number of lactic bacteria and a high contamination, a condition due to crude methods of drying and to the loss of vitality of the bacteria on standing.

When cultures are dried by exposure to a current of warm air there is a constant decrease in the number of bacteria, which is greatly accelerated when the moisture is reduced to about 10 per cent. When a water content below 5 per cent is reached the decrease becomes very slow.

The loss in drying is greatly reduced when the water is removed very rapidly, as by the spraying method.

Water may be removed from cultures by exposing them in a frozen condition over sulfuric acid in a vacuum approximating 0.01 mm.

Lactic cultures dried by this method are sufficiently active to curdle milk in 17 hours at 30° C. when added in the ratio of 1 part of powder to 1,000,000 parts of milk.

The total number of bacteria in a milk culture may be increased by adding dibasic potassium phosphate, but the powder made from the culture is less active than that made from unneutralized milk. The activity of the powder is not increased by neutralizing the culture with calcium carbonate before drying.

More powder may be produced at each operation of the dryer by using a culture grown in concentrated milk, and this powder is as active as that made from normal milk.

Very active dried cultures of *B. bulgaricus* may be made by the freezing-vacuum method.

Laboratory cultures may be preserved by drying small quantities in test tubes.

The nitrogen-fixing bacteria may be dried with a small loss by the freezing-vacuum method.

Yeasts evidently do not survive the process, and the powders obtained were very feeble.

The loss of activity in powders is much more rapid when the moisture content is comparatively high.

The loss in activity is very slow at 0° C. or lower, and becomes more rapid as the temperature is increased. Dried cultures of the lactic-acid bacteria held at 30° or 37° C. become inactive in a short time.

Cultures held in a vacuum retain their activity much better than cultures in an atmosphere of nitrogen or hydrogen; the most rapid loss of activity takes place in an atmosphere of oxygen or air.